A322

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 9/107, 9/51, 47/12, 47/44 — A1

(11) International Publication Number: WO 96/24332

(43) International Publication Date: 15 August 1996 (15.08.96)

(21) International Application Number: PCT/US96/01433

(22) International Filing Date: 31 January 1996 (31.01.96)

(30) Priority Data:

08/384,057	6 February 1995 (06.02.95)	US
08/388,088	14 February 1995 (14.02.95)	US

(71) Applicant: NANOSYSTEMS L.L.C. [US/US]; Building 1, 1250 South Collegeville Road, Collegeville, PA 19426 (US).

(72) Inventors: EICKHOFF, W., Mark; 1313 Rhode Island Circle, Downingtown, PA 19355 (US). MUELLER, Karl, R.; 45 Marilyn Avenue, Pexton, PA 19344 (US). ENGERS, David, A.; 480-5 Main Street, Collegeville, PA 19426 (US).

(74) Agents: WEST, Paul, B.; Ladas & Parry, 26 West 61st Street, New York, NY 10023 (US) et al.

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: FORMULATIONS OF COMPOUNDS AS NANOPARTICULATE DISPERSIONS IN DIGESTIBLE OILS OR FATTY ACIDS

(57) Abstract

Nanoparticulate crystalline drug substances formulated in an aqueous phase emulsified in oil, are able to be made at less than 1000 nm size and provide increased bioavailability and lymphatic uptake following oral administration.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	**GB**	United Kingdom	**MW**	Malawi
AT	Austria	**GE**	Georgia	**MX**	Mexico
AU	Australia	**GN**	Guinea	**NE**	Niger
BB	Barbados	**GR**	Greece	**NL**	Netherlands
BE	Belgium	**HU**	Hungary	**NO**	Norway
BF	Burkina Faso	**IE**	Ireland	**NZ**	New Zealand
BG	Bulgaria	**IT**	Italy	**PL**	Poland
BJ	Benin	**JP**	Japan	**PT**	Portugal
BR	Brazil	**KE**	Kenya	**RO**	Romania
BY	Belarus	**KG**	Kyrgystan	**RU**	Russian Federation
CA	Canada	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CF	Central African Republic			**SE**	Sweden
CG	Congo	**KR**	Republic of Korea	**SG**	Singapore
CH	Switzerland	**KZ**	Kazakhstan	**SI**	Slovenia
CI	Côte d'Ivoire	**LI**	Liechtenstein	**SK**	Slovakia
CM	Cameroon	**LK**	Sri Lanka	**SN**	Senegal
CN	China	**LR**	Liberia	**SZ**	Swaziland
CS	Czechoslovakia	**LT**	Lithuania	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**LV**	Latvia	**TJ**	Tajikistan
DK	Denmark	**MC**	Monaco	**TT**	Trinidad and Tobago
EE	Estonia	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**UG**	Uganda
FI	Finland	**ML**	Mali	**US**	United States of America
FR	France	**MN**	Mongolia	**UZ**	Uzbekistan
GA	Gabon	**MR**	Mauritania	**VN**	Viet Nam

FORMULATIONS OF COMPOUNDS AS NANOPARTICULATE DISPERSIONS IN DIGESTIBLE OILS OR FATTY ACIDS

Field of the Invention

The present invention relates to formulations of compounds as nanoparticulate aqueous dispersions emulsified in digestible oils or fatty acids with or without additional stabilizers. More particularly, the present invention increases the bioavailability of phamacological compounds and allows pharmacological compounds to be delivered directly to the lymphatic systems following oral administration.

Background of the Invention

Intestinal lymphatic uptake has long been proposed as a route for drugs to increase systemic bioavailability by avoiding first pass metabolism and hepobiliary elimination pathways following oral administration. However, no strong data in the literature exists which suggest there is an oral delivery system which actually can target this absorption pathway to any great extent. Formulation of drugs in oils and fatty acids is a traditional approach which has shown some success, but is by no means predictable. These approaches have focused on compounds with high log P and high lipid solubility, and even under these conditions results have been mixed. This approach suffers from the limitation that most compounds have limited solubility in digestible oils or fatty acids to the extent that development into a solid dosage form is not practical, that is, too large a capsule is needed to provide the dose.

Nanoparticl s, described in U.S. Patent No. 5,145,684, are particles consisting of a poorly soluble therapeutic or diagnostic agent onto which are adsorbed

a non-crosslinked surface modifier, and which have an average particle size of less than about 400 nanometers (nm).

The present invention provides improved oral bioavailability for any compound which possesses extensive first pass elimination and that can be formulated as a nanoparticulate in a digestible oil or fatty acid. It is theorized that nanoparticles are rapidly carried intact into the intestinal lymphatic ducts/vessels via the lipid transport pathway where subsequent dissolution in lymph/blood partitioning occurs. Eventually, any undissolved nanoparticulate will drain into the systemic circulation and represent a late phase delivery pathway.

Summary of the Invention

The present invention provides an orally administratable particle which consists essentially of 0.1-50% by weight of a crystalline drug substance having a solubility in water of less than 10 mg/ml. The drug substance has a non-crosslinked modifier adsorbed on the surface thereof in an amount of 0.1-20% by weight. The particles are suspended in an aqueous phase. The aqueous phase is emulsified in an oil or fatty acid. The particles maintain an effective size of less than 1000 nm.

In a preferred form of the present invention, the oil phase comprises oleic acid.

Description of the Preferred Embodiment

The present invention is based on the hypothesis that oral bioavailability can be dramatically improved for any compound which possesses extensive first pass elimination and that can be formulated as a nanoparticulate in a digestible oil or fatty acid.

The present invention can be practiced with a wide variety of crystalline materials that are

water insoluble or poorly soluble in water. As used herein, poorly soluble means that the material has a solubility in aqueous medium of less than about 10 mg/ml, and preferably of less than about 1 mg/ml. Examples of the preferred crystalline material are as follows. The therapeutic candidates include [6-methoxy-4-(1-methylethyl)-3-oxo-1,2-benzisothiazol-2-(3H)-yl] methyl 2,6-dichlorobenzoate, S,S-dioxide, described in U.S. Patent 5,128,339 (WIN 63394), cyclosporin, propanolol, antifungals, antivirals, chemotherapeutics, oligonucleotides, peptides or peptidomimetics and proteins. In addition it is believed that vaccines can also be delivered to the lymphatic system by use of the present invention. The present invention also allows imaging of the intestinal lymphatic system with X-ray or MRI agents formulated as nanoparticles in digestible oils or fatty acids. Potential imaging agents include any X-ray or MRI nanoparticulate core.

Suitable surface modifiers can preferably be selected from known organic and inorganic pharmaceutical excipeints. Such excipients include various polymers, low molecular weight oligomers, natural products and surfactants. Preferred surface modifiers include nonionic and ionic surfactants.

Representative examples of surface modifiers include gelatin, casein, lecithin (phosphatides), gum acacia, cholesterol, tragacanth, stearic acid, benzalkonium chloride, calcium stearate, glycerol monostearate, cetostearyl alcohol, cetomacrogol emulsifying wax, sorbitan esters, polyoxyethylene alkyl ethers, e.g., macrogol ethers such as cetomacrogol 1000, polyoxyethylene castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, e.g., the commercially available Tweens, polyethylene glycols, polyoxyethylene stearates, colloidal silicon dioxide, phosphates, sodium dodecylsulfate, carboxymethylcellulose calcium,

carboxymethylcellulose sodium, methylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose phthlate, microcrystalline cellulose, magnesium aluminum silicate, triethanolamine, polyvinyl alcohol, and polyvinylpyrrolidone (PVP). Most of these surface modifiers are known pharmaceutical excipients and are described in detail in the Handbook of Pharmaceutical Excipients, published jointly by the American Pharmaceutical Association and The Pharmaceutical Society of Great Britain, the Pharmaceutical Press, 1986.

Particularly preferred surface modifiers include polyvinylpyrrolidone, tyloxapol, poloxamers such as Pluronic F68 and F108, which are block copolymers of ethylene oxide and propylene oxide, and polyxamines such as Tetronic 908 (also known as Poloxamine 908), which is a tetrafunctional block copolymer derived from sequential addition of propylene oxide and ethylene oxide to ethylenediamine, available from BASF, dextran, lecithin, dialkylesters of sodium sulfosuccinic acid, such as Aerosol OT, which is a dioctyl ester of sodium sulfosuccinic acid, available from American Cyanimid, Duponol P, which is a sodium lauryl sulfate, available from DuPont, Triton X-200, which is an alkyl aryl polyether sulfonate, available from Rohm and Haas, Tween 20 and Tween 80, which are polyoxyethylene sorbitan fatty acid esters, available from ICI Specialty Chemicals; Carbowax 3550 and 934, which are polyethylene glycols available from Union Carbide; Crodesta F-110, which is a mixture of sucrose stearate and sucrose distearate, available from Croda Inc., Crodesta SL-40, which is available from Croda, Inc., and SA90HCO, which is $C_{18}H_{37}$-$CH_2(CON(CH_3)CH_2(CHOH)_4CH_2OH)_2$. Surface modifiers which have been found to be particularly useful include Tetronic 908, the Tweens, Pluronic F-68 and

polyvinylpyrrolidone. Other useful surface modifiers include:

decanoyl-N-methylglucamide;
n-decyl ß-D-glucopyranoside;
n-decyl ß-D-maltopyranoside;
n-dodecyl ß-D-glucopyranoside;
n-dodecyl ß-D-maltoside;
heptanoyl-N-methylglucamide;
n-heptyl-ß-D-glucopyranoside;
n-heptyl ß-D-thioglucoside; n-hexyl ß-D-glucopyranoside;
nonanoyl-N-methylglucamide;
n-noyl ß-D-glucopyranoside;
octanoyl-N-methylglucamide;
n-octyl-ß-D-glucopyranoside;
octyl ß-D-thioglucopyranoside; and the like.

Another useful surface modifier is tyloxapol (a nonionic liquid polymer of the alkyl aryl polyether alcohol type; also known as superinone or triton). This surface modifier is commercially available and/or can be prepared by techniques known in the art.

Another preferred surface modifier is p-isononylphenoxypoly(glycidol) also known as Olin-10G or Surfactant 10-G, is commercially available as 10G from Olin Chemicals, Stamford, Connecticut.

One preferred surface modifier is a block copolymers linked to at least one anionic group. The polymers contain at least one, and preferably two, three, four or more anionic groups per molecule. Preferred anionic groups include sulfate, sulfonate, phosphonate, phosphate and carboxylate groups. The anionic groups are covalently attached to the nonionic block copolymer. The nonionic sulfated polymeric surfactant has a molecular weight of 1,000-50,000, preferably 2,000-40,000 and more preferably 3,000-30,000. In preferred embodiments, the polymer comprises at least about 50%, and more preferably, at least about 60% by

weight of hydrophilic units, e.g., alkylene oxide units. The reason for this is that the presence of a major weight proportion of hydrophilic units confers aqueous solubility to the polymer.

A preferred class of block copolymers useful as surface modifiers herein includes sulfated block copolymers of ethylene oxide and propylene oxide. These block copolymers in an unsulfated form are commercially available as Pluronics. Specific examples of the unsulfated block copolymers include F68, F108 and F127.

Another preferred class of block copolymers useful herein include tetrafunctional block copolymers derived from sequential addition of ethylene oxide and propylene oxide to ethylene diamine. These polymers, in an unsulfated form, are commercially available as Tetronics.

The following investigation of preparing nanoparticle dispersions in non-aqueous media was completed for the elastase inhibitor WIN 63394. Oleic acid and three pharmaceutically acceptable oils, soybean oil, corn oil, and safflower seed oil were screened, with and without the addition of secondary stabilizers. Each combination was qualitatively characterized using light microscopy.

Favorable particle size reduction and particle dispersion stability were observed for WIN 63394 nanosuspensions milled with a Pluronic F127 to water ratio of 1:9 in oleic acid. Analysis of dispersions was limited by the their highly viscous nature. Dilution of soybean, corn, and safflower seed oil dispersions stabilized with Pluronic F127 to improve contrast between milled particles and the aqueous and non-aqueous was not effective. A description of the methods and procedures used for media conditioning, product recovery and qualitative microscopic analysis are discussed below.

All experiments requiring milling were completed in a dispersion mill. A 25 ml volume of dispersion was milled using 42.0 g of 0.5 mm acid washed glass beads. At the conclusion of the milling period, vacuum filtration was used to recover the product dispersion.

A Leitz Diaplan microscope with a PL Fluotar 100/1.32 oil object was used to make qualitative observations of the nanoparticle suspension character and estimate particle size of the product dispersions. Particle size distributions could not be quantitatively determined for dispersions in complex media such as oleic acid or oil, using traditional light scattering measurement methods, such as the Microtrac UPA, due to the viscosity and the refractive characteristics of the samples. A Sony color video camera printer was fitted to the microscope and allowed a hard copy micrograph of each sample to be generated for future reference.

The resolution of sample was limited due to the microscope power and the sample character. Dilution of each dispersion was completed using the respective oleic acid or oil medium to improve the contrast between particles and emulsion droplets. Dispersions milled in oleic acid/oil were diluted 1:2 in oleic acid/oil, respectively. This technique increased the resolution of the drug particles for dispersions milled in oleic acid. However, those suspensions milled in oils were unable to be diluted.

Example 1

Eight stabilizer systems were screened to identify a potential stabilizer for milling WIN 63394 in oleic acid and was milled four hours. Each nanoparticulate dispersion contained 222.5 mg of WIN 63394 (1%) in a measured amount of stabilizer in 22.25 g oleic acid with 42.0g of 0.5 mm acid washed glass beads. Table I outlines materials and stabilizers used to mill WIN 63394 in oleic acid.

Table I

Material	Grade	Source
WIN 63394	-	Sterling-Winthrop
Oleic Acid	NF	Spectrum
Stabilizer		
Tween 80	Reagent	Sigma
SPAN 80	Reagent	ICI
Tyloxapol	Reagent	Sigma
Pluronic F68	NF	BASF
Pluronic F127	NF	BASF
Pluronic L122	NF	BASF
Propylene glycol	Reagent	Aldrich

A description of the trials completed using Example 1 stabilizers and the micrographs for each nanosuspension is found in Table II

Table II: Description of WIN 63394 Dispersions Milled In Oleic Acid

Trial	Stabilizer	Amount (% Total)
1	Tween 80	0.25ml Tween 80 (1.0%)
2	SPAN 80	0.25ml SPAN 80 (1.0%)
3	Tyloxapol	0.25ml Tyloxapol (1.0%)
4	H_2O Pluronic F68	1.25ml H_2O (5.0%) 250mg F68 (1.0%)
5	H_2O Pluronic L122	1.25ml H_2O (5.0%) 250mg L122 (1.0%)
6	H_2O Pluronic F127	1.25ml H_2O (5.0%) 250mg F127 (1.0%)
7	Propylene glycol	6.25ml (25%)
8	50% NaOH solution	12.5ul (0.2%)
9	H_2O Pluronic F127	1.25ml H_2O (5.0%) 250mg F127 (1.0%)

* - Trial 9 was milled without WIN 63394 as a control for Trial 6.

In Table II, trials 1-8, WIN 63394 was milled in oleic acid at low solids concentrations. Trial 9 was used as a control for trial 6, which showed favorable particle size reduction of less than 1000 nanometers and good particle dispersion. In trial 8 WIN 63394 was milled without stabilizer for 3 hours and 12.5 µl 50% NaOH solution was added at 3 hours and milled for the final hour.

Good particle size reduction and stability observed in trial 6. That is, 5% H_2O, 1% Pluronic F127 in oleic

acid. In all other trials, 1-5, 7 and 8, agglomeration of drug substance was observ d. The stabilizer system of Pluronic F127 in water and oleic acid and increased WIN 63394 concentrations was investigated in Example 2.

Example 2

Trials 10-12 were completed using solid stock Pluronic F127. A 10% Pluronic F127 solution was added to trial 13. Trials 14 and 15 were milled in oleic acid as controls for trial 13, trial 14 was milled without WIN 63394 and trial 15 was milled without the addition of Pluronic F127-H2O stabilizer. A description of the trials completed are found in Table III.

Table III: Description of WIN 63394 Dispersions Milled in Oleic Acid at Increased Solids Concentration

Trial	% WIN 63394	Stabilizer (F127:H_2O Ratio)	Water	Oleic Acid
10	10.0%	0.75g F127 (dry) (1:5)	15.0%	18.6ml
11	15.0%	0.75g F127 (dry) (1:5)	15.0%	17.5ml
12	20.0%	1.0g F127 (dry) (1:5)	20.0%	15.0ml
13	10.0%	7.5ml-10% F127 soln (1:9)	-	15.0ml
14	-	7.5ml-10% F127 soln (1:9)	-	17.5ml
15	10.0%	-	-	22.5ml

The results of experiments described in Example 2 rev aled that at increased solid concentrations, i.e. 20%, dispersion viscosity is increased. As a result, milling efficiency was significantly reduced and the temperature of the suspension during milling increased dramatically. Trial 12 was discontinued after 30 minutes for these reasons. Comparison of Trials 13 and 14 is difficult due to the resolution of the samples. However, minimal agglomeration is observed in Trial 13 when diluted in 2 parts oleic acid. Trial 15 shows significant hard agglomeration in both diluted and undiluted samples.

Example 3

In addition to the dispersions milled in oleic acid, an investigation of soybean, corn, and safflower seed oil was conducted. Again, dispersions were milled using 42g of0.5 mm acid washed glass beads as the milling agent. Table IV lists the materials used for these oil milling Trials.

Table IV: Materials Used for Screening of Milling Oil Medium

Materials	Grade	Source
WIN 63394	-	-
Soybean Oil	Reagent	Sigma
Corn Oil	Reagent	Sigma
Safflower Seed Oil	Reagent	Sigma

Based on the favorable results in Trial 6, 5% H_2O-1% Pluronic F127 in oleic acid, 7.5 ml-10% Pluronic F127 solution was added to each oil medium. Controlled dispersions without stabilizer, Trials 16-18, and dispersions with stabilizer and without WIN 63394,

trials 20, 22 and 24, were completed to distinguish between drug particles and oth r components of the emulsion suspension. A description of WIN 63394 dispersions milled in oil mediums with Pluronic F127 is found in Table V.

Table V: Description of WIN 63394 Dispersions Milled in Oil Mediums

Trial	Stabilizer	% WIN 63394	Medium/Amount [ml]
16	-	3.0%	Soybean oil/ 24.25ml
17	-	3.0%	Corn oil/ 24.25ml
18	-	3.0%	Safflower seed oil/ 24.25ml
19	7.5ml-10% F127	3.0%	Soybean oil/ 16.75ml
20	7.5ml-10% F127	-	Soybean oil/ 16.75ml
21	7.5ml-10% F127	3.0%	Corn oil/ 16.75ml
22	7.5ml-10% F127	-	Corn oil/ 16.75ml
23	7.5ml-10% F127	3.0%	Safflower seed oil/ 16.75ml
24	7.5ml-10% F127	-	Safflower seed oil/ 16.75ml

Micrographs of the diluted samples from Trials 16-18 showed minimal particle agglomeration. However, as was observed in micrographs of the samples in oleic acid, resolution in between the components in the dispersion was limited. Samples from the trials milled at low solids concentrations, Trials 19, 21 and 23 were observed to have the particles residing within large water droplets. Control Trials 20 and 22 formed stable emulsions while interconnected water droplets were observed in Trial 24. All attempts to dilute the samples in their respective oil medium were unsuccessful.

Example 4

An attempt was made to optimize the Pluronic F127 to water ratio which provides a stable emulsion in oleic acid. The results of this evaluation are described below. Pluronic F127 and water were combined with 10 ml of oleic acid in 20 ml borosilicate glass vials. The vials were placed on a shaker for one hour at 400 rpm at 37 C. Qualitative analysis was completed using photomicroscopy to assess physical stability of each emulsion suspension immediately after shaking and after setting on a bench top for 3 days at 25 °C. The conditions of the trials are listed in Table IV.

Table VI: Description of Pluronic F127/H_2O Optimization Trials

Trial	Stabilizer (F127:H_2O Ratio)	Water	Oleic Acid
1	1ml-1.0% F127 soln (1:200)	-	10ml
2	1ml-1.0% F127 soln (1:100)	-	10ml
3	1ml-5.0% F127 soln (1:20)	-	10ml
4	1ml-10% F127 soln (1:10)	-	10ml
5	10mg F127 (dry) (1:100)	1ml	10ml
6	50mg F127 (dry) (1:20)	1ml	10ml
7	100mg F127 (dry) (1:10)	1ml	10ml
8	1ml-0.5% F68 soln (1:200)	-	10ml
9	1ml-0.5% F68 soln (1:20)	-	10ml

Trials 1-4 of Example 4 resulted in milky emulsions after shaking. Trials 5-7, which introduced the Pluronic F127 as a dry material also appeared to be well dispersed upon shaking, however the micrographs revealed undissolved F127 material dispersed in the oleic acid. Trials 1-7 separated into 3 phases after 3 days, but were easily returned to a milky emulsion with gentle agitation. Large water droplets were observed in the samples from Trials 8 and 9 after shaking. After 3 days, the emulsion separated into two phases and was difficult to return to an emulsion.

The results of Example 4 demonstrate that it is possible to produce a nanoparticulate aqueous dispersion emulsified in a continuous oil or fatty acid phase. Oleic acid as the fatty acid showed the best results; however, it is anticipated that other fatty acids would also produce stable nanoparticle aqueous dispersion emulsions.

The invention has been described in detail with particular reference to the preferred embodiments thereof, but it will be understood that variations and modifications can be affected within the spirit and scope of the invention.

What is Claimed is:

1. Particles consisting essentially of 0.1-50.0% by weight of a crystalline drug substance having a solubility in water of less than 10.0 mg/ml, said drug substance having a non-crosslinked modifier adsorbed on the surface thereof in an amount of 0.1-20% by weight, said particles suspended in an aqueous phase, the aqueous phase emulsified in a continuous oil phase, sufficient to maintain an effective particle size of less than 1000 nanometers.

2. The particles according to claim 1 wherein the oil phase comprises oleic acid.

3. The particles according to claim 1 wherein the surface modifier comprises poloxamers and water.

4. The particles according to claim 1 wherein the oil phase comprises a fatty acid.

5. The particles according to claim 1 wherein the oil phase comprises a digestible oil.

6. The particles according to claim 1 wherein the surface modifier comprises poloxamers 338.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 96/01433

A. CLASSIFICATION OF SUBJECT MATTER

A 61 K 9/107,A 61 K 9/51,A 61 K 47/12,A 61 K 47/44

According to International Patent Classification (IPC) or to both national classification and IPC6

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP, A, 0 608 207 (UCB, S.A.) 27 July 1994 (27.07.94), abstract; claims 1,5-8; page 3, lines 31-55; page 4, lines 5-15; examples 1-13. --	1,3,5, 6
X	DE, A, 2 714 065 (BOEHRINGER MANNHEIM GMBH) 12 October 1978 (12.10.78), claims 1,2,7-10; page 4, lines 1-9; page 6, lines 1-26; example 2. --	1,5
X	WO, A, 90/03 164 (PATRALAN LIMITED) 05 April 1990 (05.04.90), abstract; claims 1,4,7,8,14; examples 1,2,7,10,14,17-22;	1-6

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
03 June 1996	05.07.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016	MAZZUCCO e.h.

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	page 20, line 1 in connection with page 21, lines 11-21. --	
Y	EP, A, 0 499 299 (STERLING WINTHROP INC.) 19 August 1992 (19.08.92), claims 1,7,8,11-14; page 2, line 46 - page 3, line 37; page 4, line 13 - page 5, line 25; page 6, lines 19-26. --	1-6
Y	WO, A, 92/06 680 (CORTECS LIMITED) 30 April 1992 (30.04.92), abstract; claims 1,2,9; page 16, lines 13-25,30-32; page 12, line 24 - page 13, line 32. --	1-6
A	EP, A, 0 256 285 (BEHRINGWERKE AG) 24 February 1988 (24.02.88), abstract; claims 1-3; page 1, lines 1-48; page 3, lines 1-5. --	1,3,5, 6
A	EP, A, 0 315 079 (NIPPON SHINYAKU COMPANY) 28 October 1988 (28.10.88), claims 1-7; examples 1,3,6. ----	1,5

ANHANG	ANNEX	ANNEXE
zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.	to the International Search Report to the International Patent Application No.	au rapport de recherche international relatif à la demande de brevet international n°

PCT/US 96/01433 SAE 126904

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.	This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.	La presénte annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les reseignements fournis sont donnés à titre indicatif et n'engagent pas la responsibilité de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
EP A1 606207	27-07-94	AU A1 53097/94	21-07-94
		CA AA 2113243	19-07-94
		FI A0 940115	11-01-94
		FI A 940115	19-07-94
		GB A0 9300975	10-03-93
		HU A0 9400107	30-05-94
		HU A2 67213	28-03-95
		JP A2 6256172	13-09-94
		NO A0 940111	12-01-94
		NO A 940111	19-07-94
		NZ A 250673	25-11-94
		PL A1 301841	25-07-94
		US A 5500224	19-03-96
		ZA A 9400205	22-08-94
DE A1 2714065	12-10-78	AT A 2201/78	15-09-80
		AT B 361632	25-03-81
		AU A1 34510/78	04-10-79
		AU B2 513653	11-12-80
		BE A1 865426	29-09-78
		CA A1 1093966	20-01-81
		ES A1 468301	16-11-79
		FR A1 2385395	27-10-78
		FR B1 2385395	12-06-81
		GB A 1567890	21-05-80
		JP A2 53121920	24-10-78
		NL A 7803143	03-10-78
		SE A 7803478	01-10-78
		US A 4191772	04-03-80
		ZA A 7801773	23-04-79
WO A2 9003164	05-04-90	AT E 98480	15-01-94
		AU A1 42432/89	05-04-90
		AU A1 43417/89	18-04-90
		AU B2 625498	16-07-92
		DD A5 300405	11-06-92
		DE C0 68911473	27-01-94
		DE T2 68911473	05-05-94
		DK A0 4819/89	29-09-89
		DK A 4819/89	30-03-90
		EP A2 366277	02-05-90
		EP A3 366277	28-11-90
		EP B1 366277	15-12-93
		ES T3 2060785	01-12-94
		FI A0 894637	29-09-89
		FI A 894637	30-03-90
		GB A0 8822857	02-11-88
		HU A2 54033	28-01-91
		JP A2 2218609	31-08-90
		NO A0 893896	29-09-89
		NO A 893896	30-03-90
		NZ A 230838	26-03-91
		PL B1 163635	29-04-94
		PT A 91850	30-03-90
		WO A3 9003164	14-06-90
		ZA A 8907437	26-06-91
EP A2 499299	19-08-92	AU A1 10145/92	30-07-92
		AU A1 10147/92	30-07-92
		AU B2 642066	07-10-93
		AU B2 654836	24-11-94
		EP A2 498482	13-08-92
		EP A3 498483	03-03-93
		EP A3 499299	03-03-93
		FI A0 920321	24-01-92
		FI A0 920322	24-01-92
		FI A 920321	26-07-92
		FI A 920322	26-07-92
		HU A0 9200226	28-04-92
		HU A0 9200227	28-04-92
		HU A2 60635	28-10-92

		HU A2 62462	28-05-93
		HU B 208497	[illegible]-11-93
		IL A0 100754	06-05-92
		IL A0 100755	08-05-92
		IL A1 100755	08-12-95
		JP A2 4295430	20-10-92
		JP A2 4317053	05-11-92
		MX A1 9200291	01-11-92
		MX A1 9200291	01-11-92
		MX A1 9200291	01-12-92
		MX A1 9200292	01-12-92
		NO A0 920333	24-01-92
		NO A0 920334	24-01-92
		NO A 920333	27-07-92
		NO A 920334	27-07-92
		NZ A 241361	25-06-93
		NZ A 241362	25-06-93
		US A 5145684	08-09-92
		US A 5318767	07-06-94
		US A 5399363	21-03-95
		US A 5451398	19-09-95
		US A 5494683	27-02-96
		AU A1 41560/93	06-01-94
		CA AA 2098243	03-01-94
		CN A 1084391	30-03-94
		CZ A3 9301316	16-03-94
		EP A1 577215	05-01-94
		FI A0 933040	01-07-93
		FI A 933040	03-01-94
		HU A0 9301917	28-09-93
		HU A2 64832	28-03-94
		IL A0 106198	20-10-93
		JP A2 7165562	27-06-95
		NO A0 932403	01-07-93
		NO A 932403	03-01-94
		NZ A 248042	26-10-94
		SK A3 681/93	02-02-94
WO A1 9206680	30-04-92	AT E 122881	15-06-95
		AU A1 87315/91	20-05-92
		AU B2 656924	23-02-95
		BG A 97644	25-04-94
		CZ A3 9300641	19-01-94
		DE C0 69110052	29-06-95
		DE T2 69110052	05-10-95
		EP A1 553178	04-08-93
		EP B1 553178	24-05-95
		ES T3 2073177	01-08-95
		FI A 931745	16-04-93
		FI A0 931745	16-04-93
		GB A0 9022788	05-12-90
		HU A0 9301121	28-07-93
		HU A2 64215	28-12-93
		JP T2 6502143	10-03-94
		NO A 931410	16-04-93
		NO A0 931410	16-04-93
		US A 5391377	21-02-95
EP A1 256285	24-02-88	AT E 61730	15-04-91
		AU A1 75556/87	14-01-88
		AU B2 597688	07-06-90
		CA A1 1302257	02-06-92
		DE A1 3627376	21-01-88
		DE C0 [illegible]	[illegible]-04-91
		DK A0 3557/87	10-07-87
		DK A 3557/87	[illegible]-01-88
		EP B1 256285	[illegible]
		ES T3 2022214	[illegible]
		FI A0 873044	[illegible]-07-87
		FI A 873044	[illegible]-88
		GR T3 [illegible]	[illegible]
		[illegible]	[illegible]
		[illegible]	[illegible]
		PT [illegible]	[illegible]
		US A 4[illegible]45[illegible]	[illegible]
		ZA A 8705044	[illegible]-88
EP A1 315079	10-05-89	AT E [illegible]9031	[illegible]
		AU A1 24[illegible]/88	[illegible]
		AU B2 [illegible]	[illegible]
		BE AF [illegible]	[illegible]
		CA A1 [illegible]	[illegible]
		CH A [illegible]7605	[illegible]
		CN A [illegible]	[illegible]
		DE C0 [illegible]	[illegible]
		DE T[illegible] [illegible]	[illegible]
		EP B1 [illegible]	[illegible]
		ES T[illegible] [illegible]	[illegible]
		FI A [illegible]	[illegible]
		FI B [illegible]	[illegible]
		GB A [illegible]	[illegible]
		GB [illegible] [illegible]	[illegible]
		GR [illegible] [illegible]	[illegible]
		IL A0 [illegible]8076	[illegible]
		IL A1 [illegible]8076	14-[illegible]
		IT A0 8648502	2[illegible]-10-86

IT	A	1224596	04-10-90
NL	A	8802657	16-05-89
JP	A2	2000203	08-01-90
JP	B4	7098740	25-10-95